

CONTRACT REPORT NO. DSA85-00241



ACIDOPHILIC AQUATIC MOSSES AS BIOLOGICAL POLISHING AGENTS

BY

M. KALIN
AND
R. BUGGELN

BOOJUM RESEARCH LIMITED

FOR

DR. RON MCCREADY
CANMET, ENERGY, MINES AND RESOURCES
OTTAWA, ONTARIO

FINAL REPORT

IN FULFILLMENT OF DSS CONTRACT NO. 03SQ.23440-5-9236

MARCH 1986

MICROMEDIA
MAY 3 8 1986

ACIDOPHILIC AQUATIC MOSSES AS BIOLOGICAL POLISHING AGENTS

BY

M. KALIN
AND
R. BUGGELN

BOOJUM RESEARCH LIMITED

FOR

DR. RON McCREADY
CANMET, ENERGY, MINES AND RESOURCES
OTTAWA, ONTARIO

FINAL REPORT

IN FULFILLMENT OF DSS CONTRACT NO.03SQ.23440-5-9236

MARCH 1986

SUMMARY

Aquatic vascular and non-vascular plants have been reported to have the ability to concentrate and/or adsorb hazardous substances from waste streams. Their ability to improve acid coal mine drainage has been documented by several workers.

Cation exchange and binding capacities of mosses are well known and their potential function in acid seepages is investigated in this report where two extremely acidophilic moss species, Drepanocladus fluitans and Sphagnum riparium, are considered. These species are known to colonize inactive tailings ponds in the Elliot Lake (Ontario, Canada) and Uranium City (Saskatchewan, Canada) areas which have pH values of 2.5 to 3.0.

Growth experiments were conducted in the laboratory in acid seepage waters to obtain growth rates and biomass volumes. Cation exchange experiments were carried out using acid seepage water and mosses of varying degrees of cleanliness.

Growth rates were similar for both species with 3 cm.month^{-1} shoot increase. However, due to the differences in morphology and growth habit of these two species, the biomass (expressed on a dry matter basis as $\text{g/m}^2/\text{month}$) is 2.1 for D. fluitans and 17.2 for S. riparium. Under field conditions, with optimal light and temperature, it can be expected that the dry matter production is between 20 and $170 \text{ g/m}^2/\text{month}$.

The cation exchange experiments indicated that 1 g (dry weight) of moss can remove 3 ug of Ni from 100 mLs of seepage water with concentrations of

0.1 mg/L of nickel. Similar amounts of removal were observed with Cu and Mg. Acid seepage water supplemented with higher iron concentrations (up to 36 mg/L) than in the seepage creek (0.03 mg/L) inhibited metal uptake as well as cation exchange, suggesting the possibility of mass action competition effects from Mn.

Clearly, high flow rates of acid seepages cannot be realistically treated by aquatic mosses alone. However, mosses can serve a useful function in areas with reduced flow rates, and in ponds with long retention times, where their adsorptive and cation exchange abilities will improve the water quality slowly over the long-term, i.e. useful for close-out situations. In addition, their ability to act as nucleation sites for iron-hydroxide precipitates, which were found to contain appreciable concentrations of Ni (up to about 80 ug/g dry weight) and other metals can also be significant.

It can be concluded that although the cation exchange capacity of acidophilic, aquatic mosses alone will not be sufficient for a biological polishing process, these mosses can serve other useful functions such as acting as nucleation sites for adsorption and precipitation of contaminants.

SOMMAIRE

Les plantes aquatiques vasculaires et non-vasculaires s'avèrent avoir la capacité de concentrer ou d'absorber des substances dangereuses contenues dans les effluents résiduels. Plusieurs chercheurs ont étudié et documenté la capacité qu'ont ces plantes à améliorer les effluents acides des mines de charbon.

La capacité d'échange de cations et de liaison qu'ont les mousses sont des phénomènes bien connus dont ce rapport étudie le potentiel pour le traitement des suintements acides, en prenant spécifiquement pour exemple deux espèces extrêmement acidophiles: Drepanoladus fluitans et Spagnum riparium. On sait que ces espèces colonisent des bassins d'effluents miniers inactifs à Elliot Lake (Ontario, Canada) et à Uranium City (Saskatchewan, Canada), avec une échelle des pH allant de 2,5 à 3,0.

Nous avons procédé à des expériences de croissance en laboratoire sur des échantillons de suintements acides, pour déterminer les taux de croissance et les volumes de biomasse. Nous avons également fait des expériences concernant les échanges de cations dans des suintements acides avec des mousses de propreté diverse.

Les taux de croissance se sont révélés les mêmes pour les deux espèces, soit 3 cm/mois de pousse. Toutefois, en raison de la différence de morphologie et de schéma de croissance entre les deux espèces, la biomasse (exprimée en g/m²/mois de matière sèche) est de 2,1 pour D. fluitans et de 17,2 pour S. riparium. Sur le terrain, dans des conditions optimales de lumière et de température, on peut compter sur une production de matière sèche allant de 20 à 170 g/m²/mois.

Les expériences d'échange de cations indiquent que 1 gramme (poids sec) de mousse peut éliminer 3 ug de Ni dans 100 ml de suintements ayant une concentration de 0,1 mg/l en nickel. On a constaté des taux similaires d'élimination avec Cu et Mg. Dans des suintements acides ayant une concentration plus élevée en fer (jusqu'à 36mg/l) que l'effluent de drainage (0,03 mg/l), l'absorption de métal est freinée de même que l'est l'échange de cations, ce qui semble indiquer des effets concurrentiels de masse de Mn.

De toute évidence, on ne peut pas envisager réalistement de traiter des suintements acides à débit élevé avec seulement des mousses aquatiques. Toutefois, les mousses peuvent jouer un rôle utile dans les régions à débit réduit et dans les bassins à longue rétention, où leur capacité d'absorption et d'échange des cations peut améliorer lentement la qualité des eaux, à long terme, par exemple lors de la clôture de complexes miniers. De plus, elles présentent une capacité appréciable de nucléation pour les précipités d'hydroxydes de fer ayant des concentrations significatives de Ni (jusqu'à environ 80 ug/g de matière sèche) ou d'autres métaux

On conclut donc que, même si la capacité d'échange des cations des mousses aquatiques acidophiles ne suffit pas, à elle seule, à assurer un polissage biologique, ces mousses peuvent remplir d'autres fonctions utiles, notamment agir comme site de nucléation pour l'absorption et la précipitation des polluants.

ACKNOWLEDGEMENTS

We are indebted to our Scientific Authority, R. McCready, from CANMET, for his encouragement and support during this project.

We are grateful to A. Vivyurka of Rio Algom Ltd. for assistance in arranging the collection of the moss, and to Inco Ltd. - Sudbury Operations for access to an acid seepage. We would like to thank R. Kean of the Ontario Research Foundation for his patience with the analysis of the samples.

Finally, we want to thank other members of the Boojum staff for their co-operation and help in completing this project, especially M. Olaveson for assistance in the preparation of figures and production of the final report.

TABLE OF CONTENTS

	Page
SUMMARY.....	i
ACKNOWLEDGEMENTS.....	iv
List of Tables.....	vi
List of Figures.....	vi
1.0. INTRODUCTION.....	1
1.1. Objective.....	2
2.0. MATERIALS AND METHODS.....	3
2.1. Moss Collection and Handling.....	3
2.2. Growth Experiments.....	3
2.3. Ion Exchange Experiments.....	5
2.4. Analytical Methods.....	7
3.0. RESULTS.....	7
3.1. Growth Rates of <u>S. riparium</u> and <u>D. fluitans</u>	7
3.2. Metal Analyses of <u>S. riparium</u> and <u>D. fluitans</u>	9
3.3. Estimation of Seepage Creek Flow Rate.....	14
3.4. Calculation of the Biological Polishing Capacity of <u>S. riparium</u> in Acid Seepage Creek.....	14
4.0. DISCUSSION.....	17
4.1. Methodology.....	17
4.2. The Potential Role of Mosses in Acid Mine Drainage.....	19
5.0. REFERENCES.....	22

LIST OF TABLES

	Page
1 Effect of cleaning on the metal content of the moss, <u>Sphagnum riparium</u> , following equilibration with acid mine drainage creek water.....	10
2 Effect of cleaning on the metal content of the moss, <u>Drepanocladus fluitans</u> , following equilibration with acid acid mine drainage creek water.....	11
3 Comparison of Ca, Fe, Mg, Mn, and Ni concentrations in the mosses, <u>Sphagnum riparium</u> and <u>Drepanocladus</u> <u>fluitans</u> ..	13
4 Cation adsorption by <u>Sphagnum riparium</u> with competition from iron (added as FeCl_3).....	15

LIST OF FIGURES

	Page
1 Photograph showing the set-up of the moss growth experiment with <u>Sphagnum riparium</u> on the right and <u>Drepanocladus fluitans</u> on the left.....	4
2 Growth rates of acidophilic aquatic mosses investigated.....	8

1.0. INTRODUCTION

The "passive polishing" role of aquatic plants (vascular plants and algae) was recognized by Jennett and co-workers in the late 1970's. They constructed a meander leading from a lead mine tailings pond. The water leaving the pond exceeded the potability standards for both lead and zinc. Water passing through the meander, which had become populated with plants following an unspecified lag time after its construction, emerged with Zn and Pb concentrations below the maximum acceptable potability standards (cf. Hasset et al., 1980). This study appears to be one of the earliest and clearest demonstrations of the practical role of aquatic plants in metal removal from contaminated mine waters - an empirical "black box" which worked!

The cation-binding capacity of mosses is well known. Mosses have been used to monitor atmospheric deposition patterns to the earth's surface (e.g. Percy, 1983) and there have been a number of studies in which aquatic mosses and liverworts have been used as bio-detectors of metal pollution in streams, e.g. Whitton et al. (1982), Say et al. (1981), and Burton and Peterson (1979). An overview of the role of polyuronic acids in the cation-binding capacity of mosses has been given by Brown (1984) (see also Smidsrod and Painter, 1984). The preferential adsorption sequence for cations has only been determined for 2 non-aquatic moss species, Hylocomium splendens Hedw., and Hypnum cupressiforme Hedw. (cf. Brown, 1984). In these mosses, the preferential affinity for divalent nickel is only exceeded by divalent Cu and Pb. The polyuronic acid nature of the cell wall components in some Sphagnum species has been positively correlated with the degree of emergence and elevation above the water table (cf. Brown, 1984).

Seepages from impounded mine tailings ponds and acid coal mine drainages are frequently contaminated with undesirable levels of metals and inexpensive treatment systems are continuously sought in order to rectify the levels of contaminants and bring them into compliance with standards for water potability. Field-scale demonstrations of the potential for Sphagnum sp. to be used in a water filtration scheme has been reported by Wieder et al. (1985), Harris et al. (1985), and Kleinmann et al. (1983), with emphasis on the removal of Fe from acid coal mine drainage water. Lang and Weider (1985) and Weider and Lang (1985) have recorded impressive field observations on Fe and SO_4^{2-} "removal" from an acid coal mine drainage percolating through a Sphagnum bog. Anaerobic conditions in the bog led to sulphur reduction and an increase in pH.

1.1. Objective

In the present paper, we investigated two aquatic mosses, Drepanocladus fluitans, and Sphagnum riparium, which might function as self-perpetuating, metal-polishing bio-adsorbents which would reduce the metal content of seepage waters emerging from an impounded mine tailings pond. Both of these species of mosses are acidophilic and are found to colonize tailings areas at pH 2.5 to 3. Estimates of moss growth rates, cation adsorption (concentration) "profiles" of the mosses equilibrated with water from an acid seepage creek and the flow rates of this seepage were employed to critically examine the bio-adsorbent potential of beds of these two aquatic mosses.

2.0. MATERIALS AND METHODS

2.1. Moss Collection and Handling

Two acidophilic, aquatic mosses, Drepanocladus fluitans (Hedw.) Warust., and Sphagnum riparium Aongstr., were collected on 13 November 1985 from a small, inactive uranium mine tailings pond located near the town of Elliot Lake, Ontario. The former moss, growing in 3 to 5 metres of water, was collected by dragging a weighted object (from a boat) along the bottom of the pond. The latter moss, growing in shallow water (< 1 m) was easily collected by hand. The pond water had a pH of 2.7.

The mosses were transported to Toronto in polyethylene bags held in coolers. On arrival at the laboratory, specimens were sorted and material suitable for the growth and cation exchange experiments was rinsed with tap water, drained, and stored (4° C) in polyethylene bags until used.

2.2. Growth Experiments

Growth experiments were set up within 3 days of collection of the mosses. 3 cm apical shoots of D. fluitans were excised from moss clumps and the basal ends were inserted into acid-washed silica sand contained in the bottom half of 9.0 cm dia. petri plates. Similarly, 1.5 cm apical segments of S. riparium were excised and inserted into the petri plate sand beds. Each petri plate held between 40 and 49 individuals of D. fluitans and 10 and 18 of S. riparium (see Fig. 1).

Two petri plates of each moss were placed in each of two aquaria and

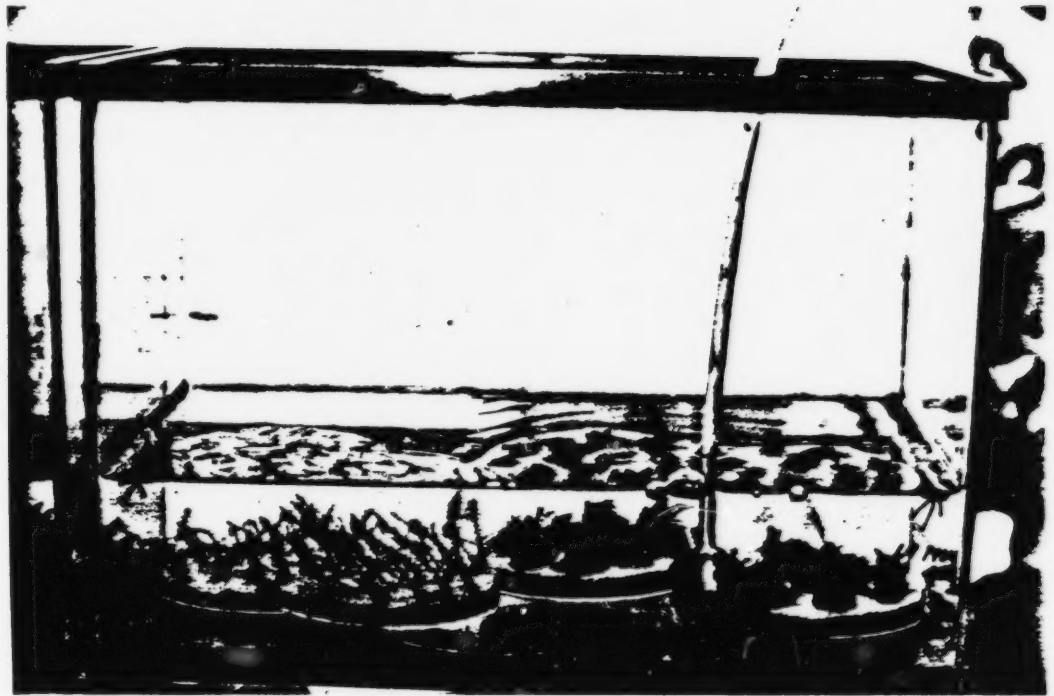


Figure 1. Photograph showing the set-up of the moss growth experiment with Sphagnum riparium on the right and Drepanocladus fluitans on the left.

covered to a depth of 6 cm with 3 L of either "control" water collected from the pond where the mosses were collected on 13 November 1985, or "experimental" water collected from an acid seepage creek (pH 4.0) in the Sudbury area on 12 November 1985. Two plates of each moss were placed in a third aquarium containing 3 L of the experimental acid seepage water supplemented with 30 mLs of KH_2PO_4 and 30 mLs of NaNO_3 stock nutrient solutions to give a final concentration of 1.3 mM and 2.9 mM, of phosphate and nitrate respectively.

Irradiance at the level of the water surface was $75\text{--}100 \text{ uE} \cdot \text{m}^2 \cdot \text{s}^{-1}$ provided by fluorescent lamps (GE "Gro and Sho"). The photoperiod was 12:12 h and the water temperature was 23°C . Air was gently bubbled through the water to provide circulation. The pH of the waters did not change over the duration of the experiment.

After 1 month, the mosses were harvested and growth was determined by measuring the increase in length of the central axis and branching axes of D. fluitans. In the case of S. riparium, little branching occurred and only the length of the central axis was measured. The new growth was cut from the plants, dried (100°C for 8 h) and weighed to obtain an average growth rate (= shoot elongation rate) and biomass increase per plant.

2.3. Ion Exchange Experiments

As the mosses were being harvested, an attempt was made to separate the collections into "more" and "less" sediment-free groups. Collection of sediment-free material was not possible. In the laboratory, several categories of moss "cleanliness" were prepared. The "less well-cleaned" group con-

sisted of moss visually judged to be free of contaminating fine reddish precipitate/sediment. In the case of S. riparium, this group contained apical tips bearing compact clusters of tightly appressed leaflets. Individual moss stems were individually shaken in deionized water to remove adhering particles. In the "well-cleaned" group, the selected pieces of moss were ground in a mortar, filtered through glass wool and then washed with several rinses of deionized water.

Unfortunately, even after this rigorous selection of material - a most tedious and time-consuming process - microscopic examination of "clean" S. riparium showed that precipitate/sediment particles were lodged in the axiles of the moss shoots, i.e. in the junction of the "leaf" and stem. In the case of S. riparium, the leaf is partially wrapped around the moss stem and forms a small cup, suitable for trapping and retaining small particles.

The hand-ground shoots of D. fluitans and S. riparium were soaked for 18-24 hours in 50 mM HNO_3 at 4° C. The mosses were rinsed with deionized water to a pH of 6.0 in order to remove eluted cations and excess acid. 10 g (fresh weight) of the acid-washed moss was equilibrated (with stirring) with 200 mLs of the acid seepage waters for 2 hours at room temperature. The moss was rinsed with deionized water to remove unadsorbed cations, then spread out on a piece of acid-washed glass and dried at 80° C overnight.

Control samples of moss were acid-washed, rinsed with deionized water and dried, as above.

2.4. Analytical Methods

The dried samples of the mosses were digested and analyzed for selected cation content by DC plasma emission spectroscopy (contractual arrangement with Dr. J. Christison, Ontario Research Foundation, Mississauga, Ontario). Seepage creek water samples were also analyzed.

3.0. RESULTS

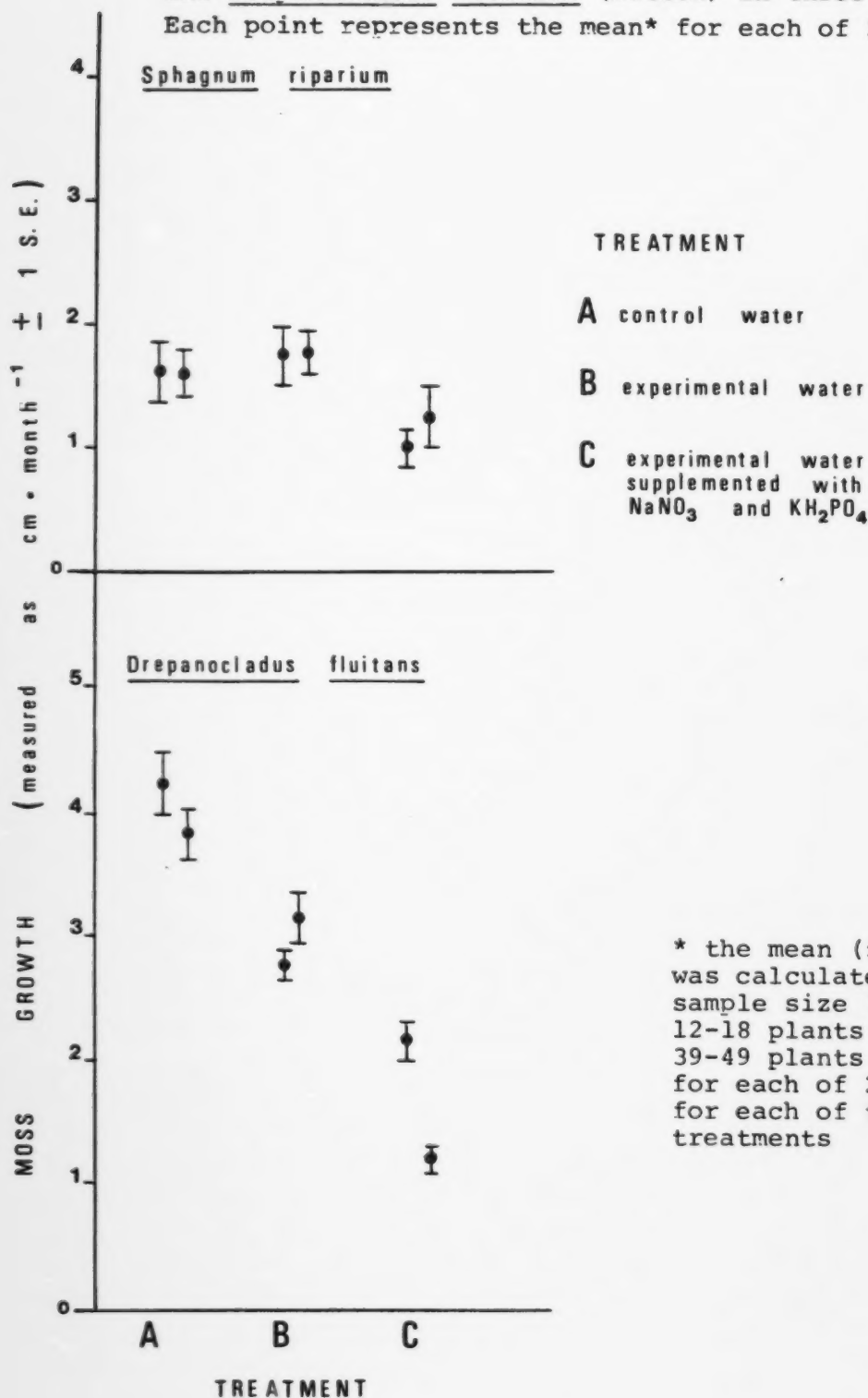
3.1. Growth Rates of *S. riparium* and *D. fluitans*

The results of the laboratory growth experiments are shown in Fig. 2. The mean (± 1 S.E.) increase in length, per plant, in one month are given for mosses growing in the control waters from the collection site and in the experimental waters from the acid seepage either amended with NO_3^- and PO_4^{3-} additions or left unamended.

The inhibiting effect of the NO_3^- and PO_4^{3-} additions is similar for both moss species which is not surprising, given the ecological niche in which these species are usually found in nature, i.e. acidic waters with high metal loadings and low N and P concentrations.

The key data are those for growth of the mosses in the unamended (experimental) acid seepage waters. The average growth rate, per plant, was similar for both species, ca. $3.0 \text{ cm.month}^{-1}$. In the case of *D. fluitans*, where 1 cm of shoot length weighs 0.1 mg dry wt, each plant produces 0.3 mg of dry matter per month. At a weight of $2.7 \text{ mg dry wt.cm}^{-1}$ shoot length for *S. riparium*, 7.3 mg of dry matter are produced by each plant per month.

Figure 2. Growth rates (expressed as $\text{cm} \cdot \text{month}^{-1}$) of two acidophilic, aquatic mosses, Sphagnum riparium (top) and Drepanocladus fluitans (bottom) in three treatments. Each point represents the mean* for each of 2 replicates.



The moss densities in the 9 cm dia. petri plates averaged 45 and 15 for D. riparium and S. fluitans, respectively, or 7,074 and 2,358 plants.m⁻². The total dry matter produced per m².month⁻¹ would therefore be in the order of 2.1 g for Drepanocladus and 17.2 g for Sphagnum.

As noted in Materials and Methods (1st Milestone Progress Report - December, 1985), the irradiance from the fluorescecent lamps, at the level of the plants in the growth experiments was 75-100 uE.m⁻².sec⁻¹. In nature, both species of moss will grow in shallow water of 1 m or less; such a shallow configuration of a meandering waterway is hypothetically envisaged as a seepage waste water treatment system which employs aquatic plants (e.g. Hassett et al., 1980). Under such conditions, solar radiation penetrating into a shallow waterway of < 0.5 m depth would be about 1500 uE.m².sec⁻¹. We would therefore expect a substantially greater growth rate of both Sphagnum and Drepanocladus under field conditions; perhaps an order of magnitude increase is not unrealistic. Based on this assumption, the dry matter productivity might be in the order of 170 and 20 g.m⁻².month⁻¹ for S. riparium and D. fluitans, respectively.

3.2. Metal Analyses of S. riparium and D. fluitans

The raw data for metal analyses of S. riparium and D. fluitans before and after exposure to the acid seepage water are given in Tables 1 and 2. These tables also indicate the effect of the cleaning treatment on these two mosses by comparing metal concentrations between less well-cleaned and well-cleaned samples of each moss. When each piece of moss was vigorously shaken in water, a considerable reduction in the concentration of metals was observed. This reduction was most noticeable for several of the divalent

Table 1. Effect of cleaning on the metal content of the moss, Sphagnum riparium, following equilibration with acid seepage water.

ELEMENT	LESS WELL-CLEANED MOSS			WELL-CLEANED MOSS	
	BEFORE ACID	AFTER ACID		BEFORE ACID	AFTER ACID
	MINE WATER	SAMPLE 1	SAMPLE 2	MINE WATER	MINE WATER *
----- ug/g -----					
Co	<0.50	<0.50	<0.50	0.67	0.71
Zn	28	12	28	5.30	7.40
Cd	<1.00	1.00	<0.50	<0.50	0.72
B	4.70	9.30	17	9.50	17
Bi	<1.00	<1.00	<1.00	4.20	<1.00
P	1130	907	1090	990	1030
Be	<0.10	<0.10	<0.10	<0.05	<0.05
Si	457	409	612	1080	970
Fe	5700	4800	5480	6640	6970
Mn	2.50	15	14	2.10	14
Ca	3060	3820	3610	145	3450
Mg	659	211	192	135	290
Cu	25	17	31	8.6	12
Al	109	129	179	230	330
V	<0.50	<0.50	<0.50	0.69	0.92
Mo	<2.50	<2.50	<2.50	<0.30	<0.30
Pb	42	42	46	14	15
Ni	12	3.80	4.00	1.10	4.20
Cr	2.40	2.50	2.40	1.30	1.30
Na	159	288	136	406	185

* meq.kg⁻¹ computed from these data = 964

cations (Ca, Cr, Cu, Fe, Mg, Mn, Ni and Zn). The results for Ca, Fe, Mg, Mn and Ni which are summarized in Table 3, are discussed in greater detail.

The summary of data for S. riparium (in Table 1) and for D. fluitans (in Table 2) illustrates an interesting feature of "less well-cleaned" and "well-cleaned" moss after exposure to the acid seepage water. Regardless of the moss pre-treatment, the individual levels of Ca, Mg and Ni (and Mn) achieved an apparent equilibrium between the moss and the seepage water. For example, the Ca in the well-cleaned moss (S. riparium -Table 1) was 145 ppm; in the less well-cleaned, 3060 ppm. After treating the moss with seepage creek water, the concentration in the well-cleaned moss was 3450 and about 3700 in the less well-cleaned material. A similar result was reported for D. fluitans (Table 2) where the Ca levels in the well-cleaned moss was 156 ppm; in the less well-cleaned, 1810 ppm. After treating the moss with seepage water, the concentration in the well-cleaned moss was about 2900 ppm while in the less well-cleaned moss, about 2700 ppm.

Manganese concentrations were similar in the acid-washed, well-cleaned and less well-cleaned moss samples, i.e. 2.1-2.5 ppm (for S. riparium - Table 3) Equivalent amounts of the element were adsorbed also from the seepage creek water, i.e. 14-15 ppm. The consistency of the Mn data, when Cu, Zn and Ni concentrations in the moss showed considerable variation, may be the result of co-precipitation of the elements which is independent of the cation-binding capacity of the moss, per se. On the other hand, the variation may be caused by the relative scale of abundance of the elements in the seepage creek water. The Mn concentration is 1 to 2 orders of magnitude greater than the levels of Cu, Zn and Ni and therefore exerts a dominant mass action competition effect for cation exchange sites on the moss.

Table 3. Comparison of Fe, Ca AND Ni in Sphagnum riparium and Drepanocladus fluitans. * All metal concentrations are expressed as ug/g. The mean (x) is given for nickel in brackets below the individual concentrations when replicate samples were analyzed.

<u>Sphagnum riparium</u>						
	LESS WELL-CLEANED		WELL-CLEANED			
	LOW Fe		LOW Fe		HIGH Fe	
	BEFORE MINE WATER	AFTER MINE WATER	BEFORE MINE WATER	AFTER MINE WATER	BEFORE MINE WATER	AFTER MINE WATER
	-----	-----	-----	-----	-----	-----
Fe	5,700	4,800; 5,480	6,640	6,970	2,620	9,850
Mg	659	211; 192	135	290	765	741
Ca	3,060	3,820; 3,610	145	3,450	490	1,070
Mn	2.5	15; 14	2.1	14	9	12
Ni	12	3.8; 4.0 (x = 3.9)	1.1	4.2	1.5	1.1
<u>Drepanocladus fluitans</u>						
	LESS WELL-CLEANED			WELL-CLEANED		
	BEFORE MINE WATER	AFTER MINE WATER		BEFORE MINE WATER	AFTER MINE WATER	
	-----	-----	-----	-----	-----	-----
Fe	12,880	12,150; 11,540; 13,590		6,040	6,540; 6,560	
Mg	325	114; 110; 117		504	408; 429	
Ca	1,810	2,920 2,520; 2,800		156	2,780; 3,020	
Mn	1.8	11; 9.0; 9.8		4.8	13; 13	
Ni	3.0	4.0; 3.7; 4.8 (x = 4.1)		1.1	2.9; 3.5 (x = 3.2)	

* Data taken from Tables 1 and 2

The role of iron in relation to metal adsorption and cation binding was briefly investigated. FeCl_3 was added to the seepage creek water increasing the iron concentration from < 0.02 to 36 ppm before treatment with the mosses. The results in Table 4 suggest that this level of Fe added to the seepage water as FeCl_3 , inhibited adsorption of Ni, Cu, Mn, Mg and Al. It should be noted that this concentration of iron is about 25 percent greater than the total soluble iron recorded in any part of the seepage creek (Kalin, 1986). At the foot of the seepage creek, where the water was collected and where moss beds could be hypothetically installed, the iron load ranged from < 0.2 to 1 ppm during the sampling season (June-November, 1985). It is doubtful that the iron levels present in the seepage would exert an inhibiting effect on Ni or other metal adsorption.

3.3. Estimation of Seepage Creek Flow Rate

The acid seepage creek used in this study as a water source is approximately 2 m wide with a water depth varying between 2.5 and 5 cm. The velocity of water flow is approximately $15\text{-}30 \text{ cm}\cdot\text{sec}^{-1}$. With these stream characteristics, the estimated volume of flow ranges between 7.5 and $15.0 \text{ cm}^3\cdot\text{sec}^{-1}$. It is likely that $7.5 \text{ L}\cdot\text{sec}^{-1}$ is a useful approximation of the flow rate.

3.4. Calculation of the Biological Polishing Capacity of *S. riparium* in the Acid Seepage Creek

In November 1985, the Ni level in the seepage creek was 0.1 ppm ($\text{mg}\cdot\text{L}^{-1}$) but values up to 0.2 ppm were recorded. To calculate nickel reduction in relation to moss growth rates and the flow rates existing in the study situa-

Table 4. Cation adsorption by Sphagnum riparium competition from iron.

ELEMENT -----	BEFORE MINE WATER -----	AFTER MINE WATER -----
Co	1.7 *	<0.5
Zn	15	31
Cd	<1	<1
B	9.2	<1
Bi	<10	<10
P	659	694
Be	<0.1	<0.1
Si	53	44
Fe	2620	9850
Mn	9	12
Ca	490	1070
Mg	765	741
Cu	19	13
Al	47	45
V	0.5	0.5
Mo	<0.3	<0.3
Pb	78	65
Ni	1.5	1.1
Cr	1.0	1.9
Na	261	393

* this value was considered unusually high and was not considered in further discussion

tion, we assumed that the present level must be reduced by 0.05 ppm. The values in Table 3 for Ni, before and after the seepage creek water has been equilibrated with S. riparium (well-cleaned), indicate that 1 kg of dry moss can adsorb 3 mg of Ni. If the Ni concentration in each litre of water must be reduced by 0.05 mg (ppm), then 1 kg of dry Sphagnum can "process" (3 mg capacity divided by 0.05 mg.L⁻¹Ni) 600 L of seepage creek water.

At an estimated flow rate of 7.5 L.sec⁻¹, 600 L of water would flow past this point in 80 seconds. At the moss density employed in the growth experiment -the plants were touching each other at their perimeters - the hypothetical production of dry Sphagnum under field conditions might be 170 kg.m⁻².month⁻¹. It would therefore require 5.9 months' worth of moss growth, i.e. 1000 g.kg divided by 170 g.m⁻².month⁻¹, to produce 1 kg of dry weight in a 1 m⁻² area to treat 80 seconds worth of seepage creek flow.

An evaluation of elemental adsorption with "well-cleaned" D. fluitans has not yet been made but on the basis of Drepanocladus growth data - together with the fact that cation exchange capacities of mosses is rather similar (ca. 0.6 to 0.8 meq.g⁻¹ dry wt) - the disparity between seepage creek flow rate (and total Ni load) and low productivity of moss biomass would be even greater than that which we have calculated to exist in the case of S. riparium.

4.0. DISCUSSION

4.1. Methodology

The objective of the present investigation was to assess the potential and practical application of two acidophilic aquatic mosses in the removal of metals especially Ni from a seepage creek; the Ni level in the creek ranged from 0.1 - 0.2 ppm. In studies of the physical-chemistry of cation binding to mosses, e.g. Gosset et al. (1986), the determination of the removal of cations from aqueous solutions is considered the most convenient experimental approach to employ. A preliminary trial using this approach showed that we were unable to detect any change in Ni content following equilibration of 100 ml of seepage creek water with 1 g (dry wt) of S. riparium for 1 hour. Only changes in Fe, Mg, Al and Ca were detected. It was therefore decided to directly analyze moss samples because preliminary trials of this technique showed more promising results.

The latter method however is not without its manifold problems which are overviewed below as a background for interpreting our results. As noted in the Introduction, a considerable number of workers have used cation content of mosses (terrestrial and aquatic) as a measure of atmospheric and aquatic pollution by heavy metals. The thoroughness of the removal of particulate matter from moss samples plagued these studies, and ours as well. No completely satisfactory physical methods have been devised (cf. Brown, 1984; Burton and Peterson, 1979) and strong acid pretreatment of the moss may decrease the cation-binding capacity through alterations in cell wall structures (Gosset et al., 1986).

Inorganic particles are digested together with moss organic matter and the chemical analysis is performed on the entire digest. The resultant spectrum of elements present in the digest contain an unknown percentage - albeit every endeavour is made to keep the percentage as small as possible - contributed by the inorganic particulates.

We found that by carefully selecting each piece of moss and vigorously shaking it in water, followed by grinding the moss in a clean mortar and then filtering through a pad of glass wool with 5-6 rinses with distilled water, a reasonably detritus-(clay)-free preparation could be obtained after mild nitric acid treatment.

Ideally speaking, and if time permitted, one might be able to raise quantities of the desired species of moss in the laboratory under conditions which would eliminate the problem of fine sediment contamination altogether. An equally insidious problem, however, would plague attempts to grow large quantities of aquatic mosses, e.g. the contaminating growth of microscopic algae which would adhere to the moss and resist cleaning measures with equal tenacity.

To develop the "optimum" acid pretreatment protocol would require extensive additional work. Some investigators studying cation binding to peat have chosen to omit an acid pretreatment step and have relied on mass action of solutions containing a single solute, e.g. Cu, Zn, etc., to provide satisfactory data on exchange capacities (e.g. Gosset et al., 1986). We could have "cleaned" up the moss with a stronger acid, but the risk of chemically altering the cation adsorption sites made such a procedure an unattractive option.

Some consistencies in the data summarized in Table 3 suggest that following a 2 hour equilibration of either S. riparium or D. fluitans with acid seepage water, where Ni is at 0.1 ppm, the mosses can adsorb a maximum of about 4 ppm Ni. This capacity results from the interaction of several physical-chemical factors, e.g. the Ni concentration in the water, concentrations of ionic species competing for the potentially Ni-adsorbing loci, etc.

4.2. The Potential Role of Mosses in an Acid Seepage Creek

It is obvious from data presented in the Results, that the slow growth rates of S. riparium and D. fluitans indicate that these mosses are inadequate for use as cation bio-adsorbents for Ni in the acid seepage creek, evaluated here. This is the myopic conclusion if one focuses exclusively on the exchange capacity of the moss cell wall and if one excludes the possibly significant role of co-precipitation of cations and particle trapping by the moss plants. Mosses would clearly be beneficial in ponds with long retention times or in seepages with slower flow rates than reported for this example. In such situations, other cleansing mechanisms attributable to the presence of mosses in addition to cation adsorption could come into play.

A case in point is the study of Zn and Pb accumulation by aquatic mosses growing in streams associated with Zn and Pb mines in Wales (Burton and Peterson, 1979). In this study, "samples were washed thoroughly in tap water to remove large particles of sand and gravel, separated into individual stems, and washed in several changes of deionized water". Mosses collected in streams close to the mines were reported to have Zn and Pb concentrations of 7,023 and 16,000 mg.kg⁻¹ dry wt. While these values are well within the

cation-binding capacity of mosses, if one takes the range of 0.6- 0.9 meq.g⁻¹ dry wt as a guideline, it is difficult to tell what percentage of the metals are adsorbed to primary exchange sites in the wall and how much is co-precipitated on Fe and Al hydroxides.

Regardless of the chemical method of adsorbing the cations, the extensive surface area of mosses enables them to adsorb metals from water. Furthermore, the mosses can filter out particulates; in the case of the acid seepage creek, examined here, these are Fe(OH)₃ precipitates with an adsorbed Ni content of about 80 ug.g⁻¹. Because of the perennial nature of both of these mosses - while they do not grow in winter, they, nevertheless, persist in the water and resume growth when water temperatures rise in the spring -their ability to entrap particulates is independent of season. This attribute places mosses in a more desirable position with respect to biological polishing functions than plants with an annual life cycle which die back and decompose in the winter. These latter plants can also act as nucleation sites for precipitation of metals but release these elements during decomposition processes.

It is interesting to note that the apparent sediment trapping ability of both S. riparium and D. fluitans was evident at the collection site, an abandoned uranium tailings pond (pH 2.7). The dredged mosses were hauled into the collection boat and frequently a considerable portion of the normally vegetative, erect portion of the moss was encased in fine, reddish precipitate/sediment. These cells were bleached white (= dead); the emergent, living portion was healthy and chlorophyllous but was frequently only a small portion of the entire plant - an indication that precipitation/ sedimentation was an ongoing process in this pond and that the moss growth rate

exceeded the rate of the precipitate/sediment accumulation.

A final note may be made on the Mn data for both D. fluitans and S. riparium. The consistency of the data has already been noted above in the Results. The removal of Mn by Sphagnum from acid coal mine drainages in the United States has been studied by Kleinmann and co-workers (1983) and by Burris et al. (1984) where Mn levels in mine discharge waters are typically 1-8 mg L⁻¹ but may be as high as 50-100 mg.L⁻¹. Gerber et al. (1985) proposed that Mn-oxidizing bacteria (as well as Fe-oxidizing ones) could "regenerate" exchange sites on Sphagnum. They discounted the addition of new adsorption sites by growth of new biomass in favour of the role of metal- oxidizing bacteria. The authors point out that more Fe and Mn are removed from water passing through a Sphagnum-dominated "volunteer" wetland than can be accounted for by simple cation exchange capacity. Hydrated iron and manganese oxides have recently been suggested as the sites for copper and zinc adsorption on submerged surfaces of inert materials (Newman et al., 1985) and mosses (Whitton et al., 1982). The Fe⁺³ ion could bind directly to cation exchange sites on a moss, thereby inhibiting the binding of Ni, as the results in Table 4 suggest, or it could adsorb to the surface of the moss as Fe(OH)₃ (which would not interfere with intra cell wall cation binding due to the molecular size of Fe(OH)₃, and adsorb cations itself.

In summary, acidophilic aquatic mosses, despite their limited cation exchange capacity coupled with their slow growth rates, are still useful as biological polishers within ecologically engineered passive polishing systems because, by using a variety of mechanisms, as discussed above, they are able to function as effective cleansing agents.

5.0. REFERENCES

- Brown, D.H. 1984. Uptake of mineral elements and their use in pollution monitoring. In (A.F. Dyer, and J.G.L. Duckett, eds). The Experimental Biology of Bryophytes. Exper. Bot. 19:229-255.
- Burton, M.A.S. and P.J. Peterson. 1979. Metal accumulation by aquatic macrophytes. Environ. Poll. 19:39-46.
- Gerber, D.W., J.E. Burris and R.W. Stone. 1985. Removal of dissolved iron and manganese ions by a Sphagnum moss system. In (Brooks, R.P., D.E. Samuel and J.B. Hill, eds) Wetlands and water management on mined lands. Proceed. Conf. 23-24 October 1985, Penn. State Univ. State College, Pa. pg. 365-372.
- Gosset, T., J.L. Trancart and D.R. Thevenot. 1986. Batch metal removal by peat: kinetics and thermodynamics. Water Res. 20:21-26.
- Harris, R.L., T.O. Tiernan, J. Hinders, J.G. Solch, B.E. Huntsman and M.L. Taylor. "Treatment of mine drainage from abandoned mines by biological iron oxidation and limestone neutralization". Peer consultants report for U.S. Bureau of Mines Contract #J0113033, 113 pp. 1984.
- Hasset, J.M., J.C. Jennett and J.E. Smith. 1980. Heavy metal accumulation by algae. In (R.N. Baker, ed). Contaminants and sediments. Ann Arbor, Michigan; Ann Arbor Publ. Inc.

- Jennett, J.C. and B.G. Wixson. 1977. In: B.G. Wixson (ed.). 1977. The Missouri Lead Studies: Final Report. Univ. of Missouri. Roela, Missouri. p. 337-340.
- Kleinmann, R.L.P., T.O. Tiernan and R.L. Harris. 1983. A low cost, low maintenance treatment system for acid mine drainage using Sphagnum moss and limestone. In (Burris, J.E. ed) Treatment of mine drainage by wetlands. Cont. #264 (1984), Dept of Biol. Penn. State Univ., Univ. Park, Pa. pg 40-44.
- Lang, G.E. and R.K. Wieder. 1985. The ability of freshwater wetlands to chemically modify acid mine drainage: mechanisms for removing hydrogen, iron and sulfate. Limnol. and Oceanogr. Abstract, 48th Annual Meeting, p. 59. Minneapolis, Minn.
- Newman, M.C., J.J. Alberts and V.A. Greenhut. 1985. Geochemical factors complicating the use of Aufwuchs to monitor bioaccumulation of arsenic, cadmium, chromium, copper and zinc. Water Res. 19:1157-1166.
- Percy, K.E. 1983. Heavy metal and sulphur concentrations in Sphagnum magellanicum Brid. in the Maritime Provinces, Canada. Water, Air and Soil Pollution 19:341-349.
- Reeder, S.W. 1979. Guidelines for surface water quality. Inland Waters Branch, Water Quality Branch, Ottawa, Canada.

Say, P.J., J.P.C. Harding and B.A. Whitton. 1981. Aquatic mosses as monitors of heavy metal contamination in the River Etherow, Great Britain. *Envir. Poll. Ser. B.* 2:295-307.

Smidsrod, O. and T.J. Painter. 1984. Contribution of carbohydrates to the cation-exchange selectivity of aquatic humus from peat-bog water. *Carbohydrate Research.* 127:267-281.

Whitton, B.A., P.J. Say and B.P. Jupp. 1982. Accumulation of zinc, cadmium, and lead by the aquatic liverwort, Scaptonia. *Envir. Poll. Ser. B.* 3:299-316.

Wieder, R.K. and G.E. Lang. 1985. Sulfur biogeochemistry in an Appalachian peatland. *Limnol. and Oceanogr.* Abstract, 48th Annual Meeting, p. 125. Minneapolis, Minn.

Wieder, R.K., G.E. Lang and A.E. Whitehouse. 1985. Metal removal in Sphagnum dominated wetlands: Experience with a man-made wetland system. *Proceed., Wetlands and Water Management on Mined Lands, Penn. State Univ., State Coll., Pa.* 23-24 October 1985, pg 353-364.